

COMPLEMENT FIXATION TECHNIQUE

ESTIMATION OF COMPLEMENT DOSES

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Before the war the usual method of employing complement for complement fixation reactions was first to determine the minimum hæmolytic dose (M.H.D.) and then choose varying amounts such as 2 M.H.D., 3 M.H.D., 4 M.H.D., and so on. The quantity selected was usually on the generous side because of the well-known thermostability of complement. During the war Richardson (1941) devised a method of preserving liquid complement which rendered it heat-stable for fixation tests. At the Venereal Disease Reference Laboratory and at the Whitechapel Clinic much work was done with this heat-stabilized complement, and the following method of titration was developed. The method of

complement titration for the Wassermann Reaction* is shown in the Figure.

Procedure.—Five sets of tubes put up in a rack. A standard rack is used and tubes of row D are placed on the right, whilst those of row E are on the left.

Row A has 10 tubes

"	B	"	5	"
"	C	"	5	"
"	D	"	7	"
"	E	"	7	"

Into each tube in row A is put 1 volume of complement diluted in tenths from 1/10 to 1/100, working from left to right; and 2 volumes of saline.

* In the case of the complement fixation test for gonorrhoea or that for amœbiasis the method is similar to that described except that one volume of serum is used.

BACK ROW

E ○ ○ ○ ○ ○ ○ ○ ○

D ○ ○ ○ ○ ○ ○ ○ ○

C ○ ○ ○ ○ ○ ○ ○

B ○ ○ ○ ○ ○ ○ ○

FRONT ROW

A ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○
 $\frac{1}{10}$ $\frac{1}{20}$ $\frac{1}{30}$ $\frac{1}{40}$ $\frac{1}{50}$ $\frac{1}{60}$ $\frac{1}{70}$ $\frac{1}{80}$ $\frac{1}{90}$ $\frac{1}{100}$
 COMPLEMENT DILUTIONS

○ COMPLETE HAEMOLYSIS
 ⊗ PARTIAL HAEMOLYSIS
 ● NO HAEMOLYSIS

1 volume complement dilution.
 $\frac{1}{2}$ volume normal serum.
 2 volumes saline.
 1 volume sensitized red blood cells.

1 volume complement dilution.
 $\frac{1}{2}$ volume strongly reacting serum.
 2 volumes saline.
 1 volume sensitized red blood cells.

1 volume complement dilution.
 $\frac{1}{2}$ volume normal serum.
 1 volume saline.
 1 volume antigen.
 1 volume sensitized red blood cells.

1 volume complement dilution.
 1 volume saline.
 1 volume antigen.
 1 volume sensitized red blood cells.

1 volume complement dilution.
 2 volumes saline.
 1 volume sensitized red blood cells.

FIGURE.—Titration of complement.

Into each tube in row B is put 1 volume of complement diluted in tenths from 1/10 to 1/50 working from left to right; 1 volume of saline; and 1 volume of antigen.

Into each tube in row C is put 1 volume of complement diluted in tenths from 1/10 to 1/50 working from left to right; 1/5 volume of normal serum; 1 volume of saline; 1 volume of antigen.

Into each tube in row D is put 1 volume of complement diluted in tenths from 1/10 to 1/70 working from left to right; 1/5 volume of strongly reacting serum; 2 volumes of saline.

Into each tube in row E is put 1 volume of complement diluted in tenths from 1/10 to 1/70 working from left to right; 1/5 volume of normal serum; 2 volumes of saline.

The volume employed in the above technique is similar to that employed in the Harrison-Wyler technique of the Wassermann reaction, that is, 0.11 ml.

After all these reagents have been added to their respective tubes they are incubated as in the test proper. For instance, in the case of the Wassermann reaction for half an hour on the bench and half an hour in a water bath at 37° C., and in the case of a gonococcal complement fixation test or a complement fixation test for *Entamoeba histolytica* for one hour in a water bath at 37° C. After incubation the rack is removed from the bath, one volume of sensitized red blood cells is added to each tube, and the rack is returned to the bath. At the end of thirty minutes the results are read.

In the latest technique the test proper is performed as a one-tube screen test and thus only two quantities of complement are required, one for the serum controls and one for the diagnostic row. The former, called the control dose, is calculated from rows D and E of the complement titration and is taken as the highest dilution of complement which shows *complete* hæmolysis in either row. This reading sails as "close to the wind" as possible on the theory that because both the control sera used in rows D and E are made up from half a dozen normal and positive sera respectively they constitute artificial average samples of sera likely to be tested, and it is reasonable to assume that their anticomplementary activity is a fair average of what might be expected. If

any test serum proved more anticomplementary than this average the serum control row of the test proper would show it.

In the Figure the dose of complement for the control row would be taken as 1 in 50.

The dose of complement used in the diagnostic row of the test proper is calculated from row C. This is done in the following manner.

The highest dilution of complement which shows *complete* hæmolysis in row C at the end of thirty minutes after the sensitized red cells have been added is multiplied

by the factor $\frac{5}{4}$. Thus, if in row C the highest dilution of complement which gives hæmolysis in thirty minutes is

1 in 40 the diagnostic dose is $\frac{1}{40} \times \frac{5}{4} = \frac{5}{160} = \frac{1}{32}$. This

25 per cent. margin allows for occasional abnormally anticomplementary sera and it has been found by experience that this extra amount of complement is sufficient to cover all contingencies except those where anticomplementary effects are also revealed by the serum control row. In fact, this calculation allows for the anticomplementary action of approximately double the amount of average serum.

This method of calculating the required doses of complement used in complement fixation tests has now been in routine use for over two years with the Wassermann reaction and the complement fixation test for gonorrhœa, and over one year with the complement fixation test for *Entamoeba histolytica*. So far there is no evidence of technical false positive reactions caused by too little complement. On the other hand it can be seen that this method, by reducing excess complement, increases the sensitivity of complement fixation tests without any loss of safety.

REFERENCE

Richardson, G. M. (1941). *Lancet*, 2, 696.